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The Journal of Parasitology

Volume 6 JUNE, 1920 Number 4

NOTES AND EXPERIMENTS ON *SARCOCYSTIS TENELLA* RAILLIET

III. IS *SARCOCYSTIS TENELLA* AN ABERRANT FORM OF ONE OF THE CNIDOSPORIDIA OF INSECTS? *

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Several years ago in the first of this series of papers the writer published some results of certain experiments which seemed to favor Darling's insect theory in regard to infection of herbivorous animals with sarcosporidia. More recently it was shown that under Wyoming conditions *Sarcocystitis tenella* is subject to seasonal infection. Notwithstanding this fact the evidence adduced in this second paper was neutral so far as the insect theory was concerned. In the present paper a report will be made of a series of experiments which were intended to show by direct means that insects were responsible for infection with *Sarcocystis tenella.* There will follow a brief discussion of the significance of these experiments. It may be stated here that the results of the experiments were negative, or rather that infection took place independent of all conditions involving insects as the original hosts of the parasite.

The outcome of certain experiments described in the first of these papers led to a favorable opinion of Darling's theory that sarcosporidia of herbivorous animals are aberrant forms of Cnidosporidia that are normally parasitic in the intestines of insects. Accordingly, a series of experiments was arranged in 1915 with the following general plan. It was thought that if the infection was due to a parasite harbored in the intestine of native insects transmission to sheep could possibly take place by two or three methods. A lamb might become infected by eating insects, by eating grass or flowers contaminated with the excreta of insects, or possibly by drinking water in which the parasites had been set free through the death of insects. If the hypothesis proved correct the second method was probably the natural method of transmission, for eating an insect would be rare and accidental, and it would seem the accidental character of the third method would not be likely to account for the almost universal infection of our range sheep. The first method, moreover, would be in principle essentially the same as the second. Consequently, all lambs with their ewes, were kept in a dry lot until they were separated into three groups on June 25. Group I, consisting of ten lambs, was allowed to graze daily from June 26 to September 18 in a pasture (1) containing a permanent pond and considerable swampy ground. Previous work had shown that a large percentage of lambs allowed to run in this pasture became infected. Group II, consisting of eight lambs, were allowed to graze in a dry pasture from June 26 to September 18. This pasture contained no water and no swampy ground. Both groups, before and after dates mentioned, were kept in a dry, bare lot, fed baled native hay, a little grain, and given city water derived from deep springs. Group II received city water all summer. Group III, consisting of nineteen lambs, was kept in a dry bare lot all summer, fed baled native hay, given some dry grain, and furnished city water to drink. The details of their further treatment are shown in Table 1. In the first column is given the number of the lamb used in each experiment. In the second column is noted briefly the feeding or other special treatment that each received; tho not given in the table, some of these lambs received an additional treatment in reference to another experiment that dealt with tapeworm infection. This second procedure was unfortunate, as results showed, and tended to involve the problem, but it was thought that if *S. tenella* was a parasite due to insects, it is logical to conclude that the secondary treatment given could have no influence on infection. Accordingly, we shall give a brief explanation and discussion of the results first in relation to the insect theory and then in relation to the secondary treatment, the nature of which is mentioned below.

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* Experiments by aid of the Adams Fund, Laboratory of Zoology and Parasitology, University of Wyoming.

From the table it will be observed that insects were fed to lambs 203, 204, 205, 214, 216, 217, 221, 256, 257 and 258; that lambs 211 and 219 drank from aquaria contaminated with insects; that lamb 209 was fed flowers from the pasture where the lambs of Group I were allowed to graze; that lamb 210 was fed grass from the same pasture. Perhaps lambs 222 and 224 should be included in this list, since pen 8, from which they were fed grass, is located in pasture 1. Search was made for insects in this pasture twice each week during the summer. A short, cold, wet season contributed to the scarcity of most groups of insects, and only certain flies and mosquitoes were abundant. So far as the insect theory is concerned, lambs 213, 225 and 227 may be regarded as control. The fourth column of the table gives the number of feedings, twenty-two being the maximum for the summer. Finally, in column five the absence of infection, or if present the apparent degree or amount, is designated. The relative amount of

TABLE 1.—OUTLINE OF EXPERIMENTS IN 1915

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
203	Flies	862	22	Mild
204	Moths, butterflies	85	16	Very light
205	Moth larvae	2	2	Light
258	Bees, wasps	32	8	Very light
256	Mosquitoes	1,098	22	None
257	Ants	22	9	None
	Beetlees	19	10	
	Dragonflies	28	9	
	Grasshoppers	9	7	
	Hemiptera	59	9	
	Spiders	33	14	
209	Flowers, pasture I	....	22	Mild
210	Grass, pasture I	....	22	Mild
211	Water, aquarium V, containing killed insects	....	9	None
214	Nosema apis (in 5 dead bees)	Very large	1	None
216	Nosema apis (in 5 dead bees)	Very large	1	Very light
217	Nosema apis (in 5 dead bees)	Very large	1	Mild
221	Nosema apis (in 3 dead bees)	Very large	1	Heavy
219	Water, aquarium IV (Nosema opis in 4 bees)	....	9	None
213	Water, aquarium II (heart muscle containing sarcocysts)	....	11	None
222	Grass, pen 8; feces and proglottids	....	10	Very light
224	Same as 222	....	9	None
225	Grass and 6 insects; from small screen cage; sod contaminated with feces and proglottids	....	3	None
227	None	....	..	None

infection expressed where infection is present should be regarded as of comparatively little value, as this is not based upon numerical data.

In order to account for the wide distribution of sarcosporidia in herbivorous animals, Darling (1915) proposed the hypothesis that herbivorous sarcosporidia were merely aberrant cnidosporidia of insects, and suggested that the habit of depositing excreta on flowers and grass by certain insects, such as bees, moths, etc., would probably be found a sufficient explanation to account for infection. If this were true, feeding the insects ought also to produce the infection. This was the basis of the series of experiments in 1915. A modification of this plan is shown in the case of lambs 211 and 219, where the lambs were made to drink from aquaria into which dead insects were thrown, and in the case of lambs 209 and 210, which were fed very considerable quantities of flowers and grass that had been exposed to insects in pasture 1. This last method would conform to normal infection according to Darling's hypothesis. A close study of the results gave no definite indication that insects either were or were not the cause of infection. For infection apparently resulted from feeding 862 flies, from feeding 85 moths and butterflies, from feeding 2 moth larvae, from feeding 32 bees and wasps, from feeding to each of three lambs 3 to 5 bees that had died with the Isle of Wight disease which is due to the Cnidosporidian, *Nosema apis,* from feeding flowers, from feeding grass, but it did not occur as the result of feeding 1,098 mosquitoes, or as the result of feeding various other insects and arachnids, including ants, beetles, bugs, dragon flies, grasshoppers and ground spiders.

infection also failed in one lamb after feeding 5 bees that had died from infection with *Nosema apis*. Both lambs exposed to aquaria containing various killed insects, including bees infected with *N. apis*, remained uninfected. If infection was due to insects it was derived from three widely different orders namely, Diptera, Lepidoptera and Hymenoptera, and it occurred as the result of eating insects or as the result of eating flowers and grass exposed to insects. One might suppose that the infection was derived from spores deposited on the hay by insects before it was baled. But considering the delicate character of the spores, this was not thought probable. Or, one might suppose the infection due to excreta of certain insects (some flies) that visit dry lots as well as pastures. One thing appeared certain: the *infection with S. tenella could not be due to N. apis.* For infection occurred independent of this parasite (lambs 203, 204, 205), and in one case it did not occur in a lamb when the parasite was known to be present.

Any other theory to account for infection, based on the special treatment received by the lambs, appeared equally unsatisfactory. Of sixteen lambs fed grass or water contaminated with feces, by the secondary treatment mentioned, eight were infected and eight not infected. Lambs 203 and 204 which had received tapeworm proglottids were both infected; the tapeworms appeared to be clean, but of course they might carry some fecal contamination. But if the infection occurred thru contamination with feces, one may well ask why were not all the lambs infected? For all lambs of Group III were exposed to the feces of the ewes running with them in the same lot, and we know that nearly 100 per cent. of our range ewes are infected with *S. tenella*. On the whole, the experiments were deemed inconclusive, since we had not been able to control the infection.

Mention should be made of the results in lambs of Groups I and II. In Group I, including the lambs which were allowed to graze in a pasture containing a pond and some swampy ground from June 26 to September 18, six out of ten lambs were found with sarcocysts. In Group II, grazed in like manner in a dry pasture, sarcocysts were found in only three out of eight lambs. These results indicated that moist or swampy pasture conditions favored infection. The significance of these results became more apparent in later work. Not much emphasis should be placed on these results, for stained sections were not prepared, and subsequent work has shown that some parasites (and probably some infections) will be overlooked unless this is done.

The unsatisfactory outcome of the year 1915 led to a new series of experiments in 1916. The failure to gain control over infection emphasized the importance of carrying out the details of the experiments in a very careful, rigid manner. Again the lambs with their accompanying ewes were divided into three groups, but the groups received somewhat different treatment. Group I, consisting of six lambs and several ewes, was allowed to graze during the summer in pasture 1. The pasture, however, had been altered in the following way: the pond previously mentioned together with a grassy slope above it was fenced off, and this fenced-off portion will hereafter be called lot III. The lambs of Group I therefore had daily access during the summer to a pasture containing considerable wet and swampy ground. Group II consisted of 22 lambs which were kept in a dry, bare lot from the time they were born until they were killed; they were given dry grain feed, watered in troughs with city water, and up until about September 15 were fed native hay kept over from the previous season; after this date these lambs were fed hay cut during the summer of 1916, since the old hay could no longer be obtained. The ewes to which these lambs belonged were kept with them in the dry lot. The individual treatment of this group of lambs is shown in Table 2, and will be mentioned later; Group III, consisting of six lambs, numbered consecutively 261 to 266, were dept in a dry, bare lot and pastured twice per week in lot III mentioned above. This lot was about 70 feet wide by 100 feet long; the pond and its borders covered most of the lower third on one side; the remainder was a dry grassy slope from 2 to 7 feet above the pond. No ewes or lambs had been on this lot since the previous season, and no ewes were allowed in lot III during 1916. So the lambs of Group III had contact with ewes only while they were in the dry lot.

TABLE 2.—INFECTION IN 1916

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
72	Grass from pasture I	....	15	Very light
73	Flowers from pasture I	....	15	Rather light
74	Flies	1,183	15	Light
75	Mosquitoes	137	7	Medium
76	Moths	65	13	None
77	Butterflies	10	5	Very light
78	Lepidoptera larvae	68	10	None
79	Bees	7	4	Light
80	Wasps	27	8	Very light
82	Ants	12	3	Light
	Beetles	482	12	
	Bugs	44	8	
	Dragonflies	37	13	
	Grasshoppers	54	14	
	Spiders	67	10	
81	Control	....	....	Very light
86	Control	....	....	None
	Control	....	....	Very light
85	Control	....	....	Very light
268	Control	....	....	Light
269	Control	....	....	Light
270	Control	....	....	None
271	Control	....	....	Light
272	Control	....	....	Light
267	Water from pond in lot III	....	12	Very light
273	Grass in pen, lot III	....	13	Medium
275	Grass in pen, lot III	....	13	Very light

Only lambs of group II are shown. See text for other groups.

An inspection of Table 2 shows a plan quite similar to that of 1915. Lamb 72 received grass and lamb 73 received flowers on fifteen different days between July 18 and September 23. At each feeding, which was done individually and by hand, the parts of flowering plants obtainable in pasture 1 were selected with the idea that they had been contaminated with the excreta of insects. With a similar idea numerous tufts of grass from different parts of the pasture were collected for each feeding of lamb 72. During the season one lamb was fed 1,183 flies; another 137 mosquitoes; another 65 moths; another 10 butterflies; another 68 lepidoptera larvae; another 7 bees; another 27 wasps, and still another was fed a miscellaneous group of insects including 12 ants, 482 beetles, 44 bugs, 37 dragonflies, 54 grasshoppers and 38 spiders. Nine lambs, numbers 81, 84, 85, 86, 268, 269, 270, 271, 272, received no special treatment, and were kept as a control. On twelve days lamb 267 was given water from the pond in lot III. On thirteen different days between July 17 and September 11 two lambs, numbered 273 and 275, were grazed in a small pen located in lot III. This pen had formerly been used as a feed pen for small pigs, but had not been used for several years; it was 10 by 12 feet in size, overgrown with grass, and no ewes or lambs had been inside of it for at least two years; it was located on the dry grassy slope of lot III some distance above the pond.

The lambs were all killed during the following winter at various times between November 3, 1916, and March 16, 1917. Material was preserved, sectioned, stained and carefully examined for parasites. The last column of Table 2 gives the general results. Group I, consisting of six lambs that grazed all summer in pasture 1, showed 100 per cent. infection. Group III likewise showed 100 per cent. infection; these lambs were grazed on fifteen different days, between July 17 and September 11, in lot III which surrounded the small pond. Of the two lambs grazed in the small pen in lot III, both were infected. So also were lambs 72, fed grass, and 73, fed flowers. Lamb 267, watered twelve times from the pond in lot III, showed a light infection. Of the nine lambs composing the control seven were infected, and the parasite was found in six out of eight of the lambs which were fed insects. Infection therefore took place independent of the control, and feeding insects did not have any discernible effect either in the number of lambs infected or in increasing the heaviness or degree of infection. A comparison with the lambs fed insects the previous year furnishes some further evidence that insects were not related to infection. For example, lamb 205 (1915) had a light infection after feeding only two moth larvae, while no infection was found in lamb 78 (1916), altho it had received 68 lepidopterous larvae, mostly those of moths. Again, no infection resulted after the feeding of 1,098 mosquitoes to lamb 256 in 1915, while feeding 137 of the same insects was followed by a medium infection in lamb 75 in 1916. The feeding of a miscellaneous group of insects and arachnids apparently produced no infection in lamb 257, and the same treatment given to another lamb the next year was followed apparently by a light infection.

From these results it was evident that infection occurred independent of the experiments that were planned to show the direct connection of insects with *S. tenella.* So if insects were responsible for infection, the parasite must be limited to such insects as were common to all of the general conditions present. Now the insects commonly present in the dry lots were limited to certain species of flies, principally houseflies and stable flies, and these were also present in the pastures and lots used. But such evidence as we had was rather opposed to the idea that these insects were responsible for the infection. For 862 flies were fed to lamb 203, and 1,183 flies were fed to lamb 74, and neither of these lambs showed more than a moderate degree of infection; while the number of flies fed included several different species, houseflies and stableflies formed a considerable part of the total number. It was seen, therefore, that we had found no evidence favoring the insect hypothesis. But since the character of the evidence was largely negative there still remained the possibility that the theory might be true.

Accordingly, in the next series of experiments it was planned to arrange the details in such a way that if infection occurred it would exclude the possibility of infection by insects. At the same time some insect and flower feeding experiments were continued. Table 3 shows the general details of these experiments. Mosquitoes were fed twice weekly to lamb 282. Flies were fed to lamb 286; bees to lamb 288; flowers to lamb 289. Flowers were also fed to lamb 287, but in this case the flowers were first heated, with the idea of killing any organisms that might be present, and then exposed to insects for several hours in a closed jar; several species of insects were included, but the common flies caught about the lots formed a large part of the number. By this means the grass was well contaminated with the feces of insects. Lamb 286 unfortunately died from an undetermined cause on July 28, after only four feedings of flies; no sarcocysts were found. The other lambs mentioned were fed twice a week as long as insects or flowers were available. Three other lambs a few days after they were born and before insects appeared, were placed in a screen cage where they were kept until long after all insects had disappeared. Of these lambs, No. 300 was fed flies twice a week, beginning July 17 and ending September 29; altogether, it was fed 1,797 flies which for the most part were caught around the dry lots. Lambs 285 and 287 were kept in the screened cage as controls and with no other treatment. Two ewes, 87, the mother of lamb 287, and 176, the mother of lambs 285 and 300, were also kept in the screened cage. During the summer a small number of houseflies gained access to the inside of the screen cage, but as these were killed as soon as discovered it is believed they did not have any effect on the experiment.

TABLE 3.—Treatment and Infection in 1917

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
282	Mosquitoes	1,809	16	Medium
286	Flies	82	4	None, died July 28
288	Bees	384	16	Light
289	Flowers	....	17	Light
297	Flowers sterilized; exposed to insects	....	17	Light
300	Kept in screen cage; fed flies	1,787	20	Light
285	Kept in screen cage; control	....	....	Light
287	Kept in screen cage; control	....	....	Heavy

Lambs 282, 286, 288, 289 and 297 were kept in a dry lot all summer.

When the lambs were killed it was found that all were infected with *S. tenella* except lamb 286 which died early. Here again it was evident that we had been unable to control infection. The two control lambs, 285 and 287, were both infected, and in these cases infection had clearly occurred without the aid of insects. In fact, lamb 287 had a heavier infection than any other lamb of the season. Again, as in previous seasons, the feeding of insects produced no noticeable effect on infection. Assuming the insect theory to be correct and that *S. tenella* is an aberrant form, one would expect that if any lambs in the screen cage were infected this would be true only of lamb 300; but such was not the case. On this theory one would expect lamb 297 to show a heavy instead of a light infection, for the sterilized plants upon a number of occasions were very generously contaminated with the feces of insects.

From the results of these three series of experiments it was believed that *Sarcocystis tenella* could not be considered an aberrant form of the Cnidosporidia, for infection apparently occurred without the presence of insects. Consequently, Darling's hypothesis on which we had been working was probably no longer tenable. However, considering the small number of lambs raised in the screened cage, it was thought desirable to obtain additional proof. So in the following year four lambs and their ewes were put in a screened cage and kept there until the lambs were killed.

Lamb 1 was born March 14 and put in the cage the following day; lamb 2 was born May 1 and put in the cage May 7; lamb 3 was born May 4 and put in the cage ten days later on May 14; lamb 6 was born May 10 and put in the cage next day. When killed, lambs 1 and 3 showed a medium degree of infection, while lambs 2 and 6 had fewer sarcocysts present.

These lambs were all put in the cage before insects appeared. From time to time one or two houseflies found a way into this cage, but as a daily search was made for such accidental visitors and they were killed as soon as found, it was believed in view of previous results that they did not have any influence on infection with *S. tenella.* On a few occasions, and for a short time only in the early summer, small gnats were observed in the cage; these had been small enough to pass thru the fine meshes of the screen. The ephemeral character of these insects would indicate that they did not harbor Cnidosporidia that would develop into *S. tenella* if transferred to the sheep. But in spite of all precautions to prevent a possible infection by insects, all four lambs were found infected when killed. Of the seven lambs that had been raised in screened cages all, or 100 per cent., were infected; this had not been true of lambs raised in dry lots outside. The percentage of infection had been increased by raising lambs in a screened cage, even tho all possible precautions were taken to protect these lambs from insects. Except for the slight possibility of infection by means of the accidental insects mentioned above, there seemed to be no longer any ground for holding to Darling's theory. There remained only one more experiment to make the proof complete. That was to raise infected lambs entirely independent of insects.

Lamb 97, born January 23, and lamb 98, born January 24, were placed in a screened cage on April 5. There were no flies in the cage at any time before the lambs were killed on July 1, and except for some small gnats and a few small mosquitoes during the latter half of June, no insects were present. As a rule, on the Laramie Plains practically no insects are present before the first of June, and they are seldom common until after the middle of June. Since it is well known that *S. tenella* requires at least four weeks to appear in the muscles after infection, it is clear that such insects as were present could have had absolutely no influence on infection. Nevertheless, lamb 98 was found lightly infected, and one of the sarcocysts was not less than two weeks old. Hence, one is forced to the conclusion that *Sarcocystis tenella* is not an aberrant form of the Cnido-sporidia of insects.

While one can now safely reject the idea that *S. tenella* is an aberrant Cnidosporidian there are still left other important questions to be answered. Does the life history of *S. tenella* require an intermediate host? By what means do lambs become infected? What is the probable life history of this parasite? These and other questions will be discussed in the next paper, after another series of experiments has been described. It is, however, desirable to mention the relation of the results found in this paper to seasonal infection. It is now apparent that the seasonal infection described in the second of this series of papers does not depend upon insects, and the conditions in some of the experiments strongly favor the idea that no intermediate host is necessary. If this is true the hypothesis that there is an infective stage in the feces of the sheep, similar to that demonstrated for *S. muris* by Nègre and Crawley, acquires added importance, and the delicate nature of the spores as noted by Fantham and Porter, coupled with adverse climatic conditions, is probably sufficient to account for seasonal infection.

It may be mentioned that lambs 97 and 98 furnished a little additional evidence on seasonal infection. Altho more than 22 weeks old when killed July 1, no sacrocysts were found in lamb 97, and the size of the sarcocysts in lamb 98 indicated that infection occurred not earlier than seven or eight weeks previous to this date. While warm periods lasting a week or two may come as early as May or the latter part of April, freezing at night and snows are common up to the first of June. Considering that these lambs were kept under conditions that always had resulted in infection in 100 per cent. of the lambs used, and remembering that infection is rather common in late spring lambs after they are 10 to 14 weeks old, it appears that the limitation of infection in lambs 97 and 98 was due to seasonal influences.

I am indebted to my assistant, Mr. E. C. O'Roke, for a considerable part of the routine work involved in the experiments mentioned in this paper. I am indebted to the Bureau of Entomology, Department of Agriculture, Washington, D. C., for the bees infected with *Nosema apis*.

SUMMARY

1. *Sarcocystis tenella* is apparently not an aberrant form of one of the Cnidosporidia of insects, for lambs become infected with this parasite without insects being present. Darling's hypothesis is therefore probably untenable.

2. It has been found that lambs are more certain of becoming infected, and that the number of parasites per unit of muscle is greater if they are kept closely confined in a screened cage than if they are allowed to run free in an open dry lot.

3. A second host other than the sheep does not seem necessary for the development of *S. tenella,* and this being true, a sexual stage of this parasite will no doubt be found in the intestine of the sheep. The method of transmission and life history will be taken up in the next paper.